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Synthesis, characterization and SEM studies of novel 1-indanyl isoniazid and hydrazide Schiff base derivatives as new anti-tubercular agents

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Abstract: The aim of study was to synthesize 1-indanyl isoniazid and sixteen other hydrazide Schiff base derivatives from 1-indanone. All synthesized derivatives were screened for the inhibitory activity against *Mycobacterium tuberculosis* on three Mycobacterial strains ATCC H37Rv, known INH-sensitive (INH-S) and INH-resistant strains (INH-R) by proportion method. The derivatives were characterized using different spectroscopic techniques such as UV-Visible, FTIR, ^{1}H NMR, and HREIMS. In addition, to gain more insight into morphology of the structures, Scanning electron microscopy (SEM) was also performed. The results revealed that 1-indanyl isoniazid derivative (UN-1) exhibited more potent and high anti-mycobacterial activity against both INH-sensitive and INH-resistant strains of *Mycobacterium tuberculosis* when compared to standard anti-tubercular drug isoniazid which might be a novel isoniazid derivative as a new anti-tubercular agent.

Keywords: 1-indanone, Isoniazid, hydrazide, Schiff base derivatives, SEM, anti-tubercular agent.

INTRODUCTION

Tuberculosis (TB) is presently regarded as the most dangerous infective disease world-wide and one of the major AIDS associated infections. At present, according to statistics, TB kills four people every minute somewhere in the world and accounts for about two million deaths per year (da Silva Lourenco *et al*., 2008). Although there are several drugs clinically used for TB treatment but the resistance problem associated with the currently available first line drugs against the virulent strains of MTB incite the researchers to design new anti TB agents on the priority basis (Wei *et al*., 2013).

The molecule, Indanone used for derivatization, is one of the attractive scaffolds in medicinal chemistry research that have already been found in many clinically used drug molecules and other diversely substituted derivatives. (Patil *et al*., 2017, Vilums *et al*., 2015) (Saha *et al*., 2014, Karthik *et al*., 2008).A careful literature survey of functional groups that could be considered as pharmacophores for anti-tubercular activities revealed that hydrazide moiety was an important pharmacophoric group common among some new anti-tubercular agents, such as salinazide and verazide. (Joshi *et al*., 2008) (Dogan *et al*., 1998) (Narasimhan *et al*., 2010). Recently, interesting anti-mycobacterial activities were reported about the guanyl hydrazones, trans-cinnamic acid hydrazides derivatives, fluorine containing hydrazones, sulfonyl-hydrazones derivatives (Oliveira *et al*., 2017). In 2008, an extended study reported a quantitative structure activity relationships (QSAR) of a large hydrazide family for the development of antitubercular compounds (Ventura and Martins, 2008).Therefore, the target compounds were rationalized so as to comprise the hydrazide pharmacophores that are believed to be responsible for the biological significance of some relevant chemotherapeutic agents. The substitution pattern of such hydrazide derivatives was carefully selected so as to confer different electronic environment to the indanyl ring.Numerous efforts have also been undertaken to develop new anti-TB agents, but no new drug has been introduced for more than 5 decades. Various derivatives of isoniazid have also been synthesized so far with the aim to develop better lead molecules for anti-tubercular drug discovery (Naeem *et al*., 2018, Ali *et al*., 2017).. Hence, improvement of INH by introducing chemical modifications in its core structure in order to enhance the biological response against Mtb and/or circumvent resistance phenomena might be an interesting area of research (Hu *et al*., 2017).

Considering these facts, we have reported the synthesis of novel 1-indanyl isoniazid derivative UN-1and sixteen other 1-indanyl hydrazide derivatives UN-2 to 17 (table 1) and attempted to screen the potential of these derivatives against sensitive and resistant strains of *Mycobacterium tuberculosis*.

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MATERIALS AND METHODS

All reagents and solvents were used of Sigma Aldrich - without further purifications. Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra (KBr discs) were run on a Shimadzu 8400 and Shimadzu Prestige-21 FT-IR spectrometer. The ^{1}H-NMR spectra were recorded in DMSO-d_6 on Bruker (Rhenis-tetten-Forchheim, Germany) AM 300 spectrometer operating at 300 and 400 MHz, using TMS as an internal standard. The electron impact mass spectra (EIMS) were determined with a Finnigan MAT-312 and a JEOL MS Route mass spectrometer. Elemental analysis (CHNS) was carried out on a Perkin Elmer 2400 Series II elemental analyzer. SEM images were recorded by Scanning Electron Microscope, Jeol Japan model no. JSM6380A. Prior to imaging, the samples were coated using an auto-coater Jeol Japan model no. JFC1500.

Synthesis

All Schiff base derivatives were synthesized by a previously reported simple method (Akhlaghi *et al*., 2014). The precursor molecule, 1-indanone (1 mmol) was stirred with few drops (2 ml) of glacial acetic acid in ethanol (20 ml) for 25-30 min and isoniazid and other respective hydrazides (1mmole) were added to it. The mixture was then refluxed for 10-12 h at 80 ^{0}C and then poured into crushed ice. The hydrazide derivatives of 1-indanone precipitated out which was filtered and recrystallized in ethanol (Scheme-1).

Anti-tubercular activity

Anti-tubercular activity was performed by using proportion method (Canetti *et al*., 1969, Canetti *et al*., 1963, Anochie *et al*., 2011). First egg-based medium Lowenstein-Jensen (LJ) was prepared by combining the liquid LJ (dissolved in glycerol and Sterile Distilled Water (SDW)) and egg-emulsion. Stock solution (1000 µg/mL) of each test compound was prepared using DMSO as a solvent which was then diluted to 100 µg/mL and 10 µg/mL using SDW and further diluted with SDW.For Drug Susceptibility Testing (DST), three strains MTB H37Rv, INH-sensitive (INH-S) and INH-resistant (INH-R) were selected. Serial dilution of each strain was performed from calibrated bacterial suspension equal to McFarland No. 1, preparing tenfold serial dilutions down to a dilution between 100 and 10000 using SDW. After one week of incubation, the inoculated media was checked for contamination and the caps were tightly closed. Slopes were checked visually for the presence of mycobacterium and followed the critical proportion of 1%. If the growth on the media containing drug is > to the growth on 10^{-4} control, the strain is considered resistant to that particular drug concentration. On the other hand, if the growth on the media containing one drug is < to the growth on 10^{-4} control, the strain is considered susceptible to that particular drug concentration.

RESULTS

All Schiff base derivatives listed in table 1 were obtained in good yield in refluxing times of 10-12 h. To the best of our knowledge, all synthesized compounds (UN-1 to 17) are herein reported for the first time and As far as we know they have never been tested against Mtb.

Spectral characterization of synthesized compounds

UN-1

Yield: 82%, IR (KBr) cm^{-1}:3197 (NH, amide), 1668.7 (C=O, amide), 1632.1 (C=N), 1525.2 (C=N), 1208.8 (C-N, amide); ^{1}H NMR (300MHz, DMSO-d_6): δ 10.83 (s, 1H, NH), 8.76 (d, 2H, J=7.6 Hz, pyridine H-3', H-5'), 7.77 (d, 2H, J = 7.8 Hz, pyridine H-2', H-6'), 7.73 (d, 1H, J = 8.0 Hz, H-7), 7.42 (t, 2H, H-5, H-6), 7.35(d, 1H, J = 7.8 Hz, H-4), 3.09 (d, 2H, J=7.2 Hz, H-2), 3.00 (d, 2H, J = 7.4 Hz, H3); EI-Mass *m/z*: 250.9 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{15}H_{13}N_3O$: 251.10 Found 251.10; Anal. Calcd. For $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72; O, 6.37; Found C, 71.69; H, 5.15; N, 16.75; O, 6.41; λ_{max} = 311 nm.

UN-2

Yield: 89%, IR (KBr) cm^{-1}: 3210 (NH, amide), 1660.7 (C=O, amide), 1627.1 (C=N, imine), 1537.5 (C=N, pyridine), 1208.3 (C-N, amide); ^{1}H NMR (400MHz, DMSO-d_6): δ 10.98 (s, 1H, NH), 9.00 (s, 1H, pyridine H-2'), 8.73 (s, 1H, pyridine C_6-H-6'), 8.20 (d, 1H, J = 7.7 Hz, pyridine H-5'), 7.73 (d, 1H, J= 7.5 Hz, pyridine H-4'), 7.55 (t, 1H, H-7), 7.41 (s , 2H, H-5, H-6), 7.33(s, H, H-4), 3.10 (m, 2H, H-2), 2.98 (m, 2H, H-3); EI-Mass *m/z*: 251.0 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{15}H_{13}N_3O$:251.10 Found 251.10; Anal. Calcd. For $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72; O, 6.37 Found: C, 71.73; H, 5.18; N, 16.76; O, 6.33; λ_{max} = 310 nm.

UN-3

Yield: 87%, IR (KBr) cm^{-1}: 3138.5 (NH, amide), 1642.9 (C=O, amide), 1537.4 (C=N, imine), 1281.9 (C-N, amide); ^{1}H NMR (300MHz, DMSO-d_6): δ 10.57 (s, 1H, NH), 7.87 (d, 2H, $J_{2',3'/6',5'}$ = 6.9 Hz, H-2', H-6'), 7.70 (s, 1H, H-7), 7.57 (m, 3H, H-3', H-4', H-5'), 7.40 (s, 2H, H-5, H-6), 7.32 (s, 1H, H-4), 3.09 (d, 2H, *J* = 7.2 Hz, H-2), 2.99 (br. s, 2H, H-3); EI-Mass *m/z*: 250.2 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{16}H_{14}N_2O$ 250.11, Found 250.10; Anal. Calcd. For $C_{16}H_{14}N_2O$: C, 76.72; H, 5.70; N, 11.12; O, 6.24. Found: C, 76.82; H, 5.60; N, 11.26; O, 6.32; λ_{max} = 306 nm.

Scheme 1: General synthetic route for the synthesis of 1-indanyl hydrazide Schiff base derivatives.

Table 1: 1-indanyl isoniazid and other hydrazide Schiff base derivatives

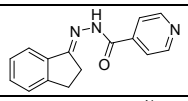
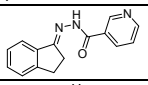
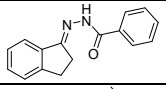
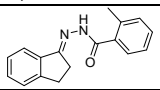
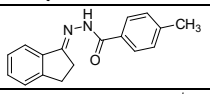
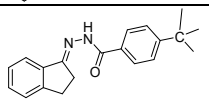
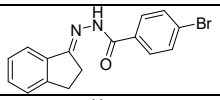
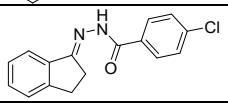
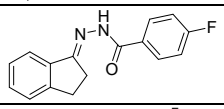
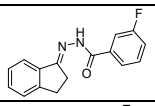
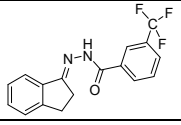
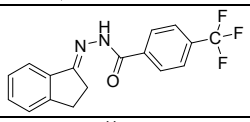
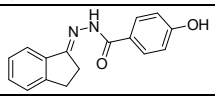
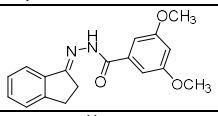
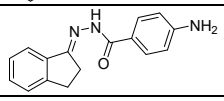
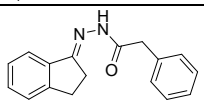
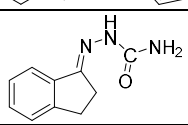
S. No	Compounds	Mol. Mass	Structure	IUPAC Name	MP (°C)
1	UN-1	251.28		Isonicotinic acid indan-1-ylidene-hydrazide	217
2	UN-2	251.28		Nicotinic acid indan-1-ylidene-hydrazide	201, *d*
3	UN-3	250.3		Benzoic acid indan-1-ylidene-hydrazide	175.6
4	UN-4	264.32		2-Methyl-benzoic acid indan-1-ylidene-hydrazide	195
5	UN-5	264.32		4-Methyl-benzoic acid indan-1-ylidene-hydrazide	189
6	UN-6	306.4		4-tert-Butyl-benzoic acid indan-1-ylidene-hydrazide	246
7	UN-7	329.19		4-Bromo-benzoic acid indan-1-ylidene-hydrazide	193
8	UN-8	284.74		4-Chloro-benzoic acid indan-1-ylidene-hydrazide	204
9	UN-9	268.20		4-Fluoro-benzoic acid indan-1-ylidene-hydrazide	190
10	UN-10	268.20		3-Fluoro-benzoic acid indan-1-ylidene-hydrazide	188
11	UN-11	318.29		3-Trifluoromethyl-benzoic acid indan-1-ylidene-hydrazide	174
12	UN-12	318.29		4-Trifluoromethyl-benzoic acid indan-1-ylidene-hydrazide	211
13	UN-13	266.29		4-Hydroxy-benzoic acid indan-1-ylidene-hydrazide	267
14	UN-14	310.35		3,5-Dimethoxy-benzoic acid indan-1-ylidene-hydrazide	192
15	UN-15	265.31		4-Amino-benzoic acid indan-1-ylidene-hydrazide	246
16	UN-16	264.38		Phenyl-acetic acid indan-1-ylidene-hydrazide	176
17	UN-17	189.21		Indan-1-ylidene-hydrazine carboxamide	244, *d*

Table 2: Comparison of antimycobacterial activity of synthesized derivatives with pure INH

Compounds	H37Rv Test concentration (µg/mL)				INH-S Test concentration (µg/mL)				INH-R Test concentration (µg/mL)			
	0.1	0.2	0.5	1.0	0.1	0.2	0.5	1.0	0.1	0.2	0.5	1.0
INH (STD)	S	S	S	S	R	S	S	S	R	R	R	R
UN-1	S	S	S	S	S	S	S	S	S	S	S	S
UN-2	R	R	R	R	R	R	R	R	R	R	R	R
UN-3	R	R	R	R	R	R	R	R	R	R	R	R
UN-4	R	R	R	R	R	R	R	R	R	R	R	R
UN-5	R	R	R	R	R	R	R	R	R	R	R	R
UN-6	R	R	R	R	R	R	R	R	R	R	R	R
UN-7	R	R	R	R	R	R	R	R	R	R	R	R
UN-8	R	R	R	R	R	R	R	R	R	R	R	R
UN-9	R	R	R	R	R	R	R	R	R	R	R	R
UN-10	R	R	R	R	R	R	R	R	R	R	R	R
UN-11	R	R	R	R	R	R	R	R	R	R	R	R
UN-12	R	R	R	R	R	R	R	R	R	R	R	R
UN-13	R	R	R	R	R	R	R	R	R	R	R	R
UN-14	R	R	R	R	R	R	R	R	R	R	R	R
UN-15	R	R	R	R	R	R	R	R	R	R	R	R
UN-16	R	R	R	R	R	R	R	R	R	R	R	R
UN-17	R	R	R	R	R	R	R	R	R	R	R	R

R = Resistant, S = Sensitive

UN-4

Yield: 81%, IR (KBr) cm^{-1}: 3196.6 (NH, amide), 1652.3 (C=O, amide), 1539.2 (C=N, imine), 1275.5 (C-N, amide); ^{1}H NMR (300MHz, DMSO-d_6): δ 8.002 (d, 1H, *J* =7.8 Hz, H-6'), 7.48 (d, 1H, J=7.5 Hz, H-7), 7.42 (m, 3H, H-3', H-4', H-5'), 7.33 (m, 3H, H-4, H-5, H-6), 3.16 (m, 2H, H-2), 2.92 (m, 2H, H-3), 2.47 (s, 3H, CH_3); EI-Mass *m/z*: 264.1 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{17}H_{16}N_2O$: 264.12 Found: 264.12; Anal. Calcd. For $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60; O, 6.05; Found: C, 77.28; H, 6.07; N, 10.64; O, 6.01; λ_{max} = 301 nm.

UN-5

Yield: 82%, IR (KBr) cm^{-1}: 3228.84 (NH, amide), 1660 (C=O, amide), 1533.41 (C=N, imine), 1276.88 (C-N, amide); ^{1}H NMR (300MHz, DMSO-d_6): δ 10.47 (s, 1H, NH), 7.78 (d, 2H, *J*=7.6 Hz, H-2', H-6'), 7.68 (s, 1H, H-7), 7.41 (d, 2H, *J*=7.7 Hz, H-3', H-5'), 7.31 (d, 3H, *J*=7.8 Hz, H-4, H-5, H-6), 3.10 (m, 2H, H-2), 2.99 (m, 2H, H-3), 2.37 (s, 3H, CH_3); EI-Mass *m/z*: 264.0 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{17}H_{16}N_2O$: 264.12 Found: 264.12; Anal. Calcd. For $C_{17}H_{16}N_2O$: C, 77.20; H, 6.15; N, 10.55; O, 6.10 Found: C, 77.25; H, 6.05; N, 10.66; O, 6.04; λ_{max} = 307 nm

UN-6

Yield: 81%, IR (KBr) cm^{-1}: 3154.8 (NH, amide), 1647.1 (C=O, amide), 1544.4 (C=N, imine), 1278.2 (C-N, amide); ^{1}H NMR (300MHz, DMSO-d_6): δ 10.48 (s, 1H, NH), 7.80 (d, 2H, *J*=7.9 Hz, H-2, H-6,), 7.68 (s, 1H, H-7), 7.52 (d, 2H, *J*=7.8 Hz, H-3, H-5), 7.34 (d, 2H, *J*=7.6 Hz, H-5, H-6), 7.30 (dd, 1H, J = 1.6 Hz, H-4), 3.10 (m, 2H, H-2), 2.98 (m, 2H, H-3), 1.31 (s, 9H, t-butyl); EI-Mass *m/z*: 306.17 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{20}H_{22}N_2O$ $[M]^+$:306.0 Found: 306.17; Anal. Calcd. For $C_{20}H_{22}N_2O$: C, 78.40; H, 7.24; N, 9.14; O, 5.22. Found: C, 78.43; H, 7.21; N, 9.16; O, 5.20; λ_{max} = 310 nm

UN-7

Yield: 79%, IR (KBr) cm^{-1}: 3205.6 (NH, amide), 1606.7 (C=O, amide), 1525.6 (C=N, imine), 1278.8 (C-N, amide), 653.8 (C-Br); ^{1}H NMR (300MHz, Me OD): δ 10.64 (s, 1H, NH), 7.98 (d, 1H, J = 7.6 Hz, H-7), 7.83 (d, 2H, J = 7.9Hz, H-2', H-6'), 7.69 (d, 2H, J = 7.8 Hz, H-3', H-5'), 7.42 (t, 2H, J = 6.7 Hz, H-5, H-6), 7.33 (t, 1H, J = 6.5 Hz, H-4), 3.19 (m, 2H, H-2), 3.03 (m, 2H, H-3); EI-Mass *m/z*: 329.2 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{16}H_{13}BrN_2O$ $[M]^+$ 329.19 Found 329.19; Anal. Calcd. For $C_{16}H_{13}BrN_2O$: C, 58.32; H, 3.98; Br, 24.31; N, 8.51; O, 4.86 Found: C, 58.38; H, 3.96; Br, 24.29; N, 8.57; O, 4.80; λ_{max} = 310 nm.

UN-8

Yield: 88%, IR (KBr) cm^{-1}: 3295.2 (NH, amide), 1659.4 (C=O, amide), 1529.7 (C=N, imine), 1270.3 (C-N, amide), 752.8 (C-Cl); ^{1}H NMR (400MHz, DMSO-d_6): δ 10.64 (s, 1H, NH), 7.89 (d, 2H, J=7.8 Hz, H-2', H-6'), 7.70 (s, 1H, H-7), 7.58 (d, 2H, J= 7.5 Hz, H-3', H-5'), 7.41 (s, 2H, H-5, H-6), 7.32 (s, 1H, H-4), 3.09 (m, 2H, H-2), 2.97 (s, 2H, H-3); EI-Mass *m/z*: 283.9 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{16}H_{13}ClN_2O$ $[M]^+$284.07 Found 284.07; Anal. Calcd. For $C_{16}H_{13}ClN_2O$: C, 67.49; H, 4.60; Cl,

12.45; N, 9.84; O, 5.62. Found: C, 67.41; H, 4.68; Cl, 12.47; N, 9.82; O, 5.62; λ_{max} = 310 nm

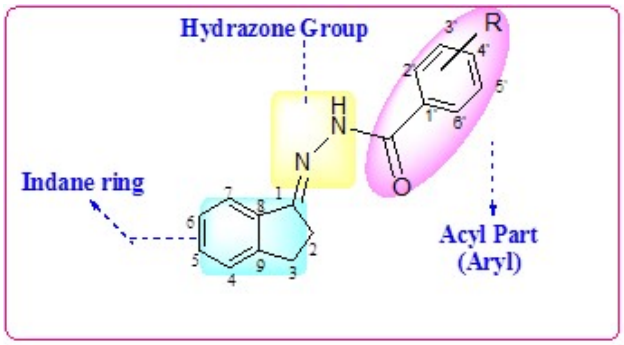


Fig. 1: General structure of 1-indanyl aryl hydrazide derivatives

UN-9

Yield: 86%, IR (KBr) cm^{-1}: 3463.3 (NH, amide), 1659.7 (C=O, amide), 1500.6 (C=N, imine), 1283.6 (C-N, amide), 1099 (C-F); ^{1}H NMR (300MHz, DMSO-d_6): δ 10.59 (s, 1H, NH), 7.96 (m, 2H, H-2', H-6'), 7.69 (s, 1H, H-7), 7.40 (s, 2H, H-3', H-5'), 7.33 (d, 3H, *J*= 8.0 Hz, H-4, H-5, H-6), 3.10 (t, 2H, H-2), 2.96 (s, 2H, H-3); EI-Mass *m/z*: 268.0 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{16}H_{13}N_2OF$ $[M]^+$: 268.10 Found 268.10; Anal. Calcd. $C_{16}H_{13}N_2OF$: C, 71.63; H, 4.88; F, 7.08; N, 10.44; O, 5.96. Found: C, C, 71.63; H, 4.91; F, 7.05; N, 10.48; O, 5.92; λ_{max} = 307 nm

UN-10

Yield: 89%, IR (KBr) cm^{-1}: 3208.1 (NH, amide), 1651(C=O, amide), 1539 (C=N, imine), 1275 (C-N, amide), 1095.4 (C-F); ^{1}H NMR (300MHz, DMSO-d_6): δ 10.65 (s, 1H, NH), 7.72 (t, 3H, H-2, H-6', H-7), 7.59 (m, 1H, C_5-H-5'), 7.41 (s, 3H, H-4', H-4, H-5), 7.33 (s, 1H, H-6), 3.10 (m, 2H, H-2), 2.98 (m, 2H, H-3); EI-Mass *m/z*: 268.0 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{16}H_{13}N_2OF$ $[M]^+$268.10 Found 268.10; Anal. Calcd. For $C_{16}H_{13}N_2OF$: C, 71.63; H, 4.88; F, 7.08; N, 10.44; O, 5.96. Found: C, 71.64; H, 4.87; F, 7.11; N, 10.41; O, 5.96; λ_{max} = 308 nm

UN-11

Yield: 80%, IR (KBr) cm^{-1}: 3207.5 (NH, amide), 1655.4 (C=O, amide), 1538.7 (C=N, imine), 1337 (C-N, amide), 1118.9 (C-F); ^{1}H NMR (300MHz, DMSO-d_6): δ 10.82 (s, 1H, NH), 8.15 (s, 2H, H-2', H-6'), 7.95 (d, 1H, J= 7.9 Hz, H-4'), 7.78 (m, 2H, H-7, H-5'), 7.43 (d, 2H, J = 7.6 Hz, H-4, H-5), 7.38 (m, 1H, H-6), 3.11 (m, 2H, H-3) 3.01 (m, 2H, H-2); EI-Mass *m/z*: 318 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{17}H_{13}N_2OF_3$ $[M]^+$ 318.09 Found 318.09; Anal. Calcd. For $C_{17}H_{13}N_2OF_3$: C, 64.15; H, 4.12; F, 17.91; N, 8.80; O, 5.03 Found: C, 64.18; H, 4.11; F, 17.89; N, 8.80; O, 5.03; λ_{max} = 310 nm

UN-12

Yield: 88.5%, IR (KBr) cm^{-1}: 3192.19 (NH, amide), 1656.85 (C=O, amide), 1546.9 (C=N, imine), 1286.5 (C-N, amide), 1332.7 and 1114.8 (C-F); ^{1}H NMR (300MHz, DMSO-d_6): δ 10.77 (s, 1H, NH), 8.06 (d, 2H, J=7.6 Hz, H-2', H-6'), 7.89 (d, 2H, J=8.1 Hz, H-3', H-5'), 7.73 (d, 1H, J=7.7 Hz, H-7), 7.41 (s, 2H, H-4, H-5), 7.33 (s, 1H, H-6), 3.11 (m, 2H, 2H-2), 2.99 (m, 2H, 2H-3); EI-Mass *m/z*: 318.0$[M]^+$; HREI-MS: *m/z* Calcd for $C_{17}H_{13}N_2OF_3$ $[M]^+$ 318.09 Found 318.09; Anal. Calcd. For $C_{17}H_{13}N_2OF_3$: C, 64.15; H, 4.12; F, 17.91; N, 8.80; O, 5.03. Found: C, 64.14; H, 4.14; F, 17.90; N, 8.83; O, 5.00; λ_{max} = 310 nm

UN-13

Yield: 80%, IR (KBr) cm^{-1}: 3109.8 (NH, amide), 1660 (C=O, amide), 1580.2 (C=N, imine), 1274.6 (C-N, amide), 1219 (C-O); ^{1}H NMR (300MHz, DMSO-d_6): δ 10.30 (s, 1H, NH), 10.02 (s, 1H, OH), 7.77 (d, 2H, *J*= 8.5 Hz, H-2', H-6'), 7.68 (d, 1H, *J*=7.2 Hz, H-7), 7.40 (overlapping multiplet, 3H, H-4, H-5, H-6), 6.85 (d, 2H, *J* = 8.4 Hz, H-3', H-5'), 3.10 (m, 2H, H-2), 2.98 (m, 2H, H-3); EI-MS *m/z*: 265.9 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{16}H_{14}N_2O_2$ $[M]^+$ 266.10 Found 266.10; Anal. Calcd. For $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.30; N, 10.52; O, 12.02 Found: C, 72.15; H, 5.31; N, 10.54; O, 12.00; λ_{max} = 310 nm

UN-14

Yield: 92%, IR (KBr) cm^{-1}: 3227.6 (NH, amide), 1657.5 (C=O, amide), 1532.4 (C=N, imine), 1352.8 (C-N, amide), 1154.5 and 1059.4 (C-O, ether); ^{1}H NMR (300MHz, DMSO-d_6): δ 10.51 (s, 1H, NH), 7.71 (d, 1H, *J* =7.7 Hz, H-7), 7.40 (s, 2H, H-4, H-5), 7.32 (s, 1H, H-6), 6.99 (d, 2H, *J*=7.9 Hz, H-2', H-6'), 6.68 (s, 1H, H-4'), 3.80 (s, 6H, OCH_3 × 2), 3.09 (m, 2H, H-2), 2.99(d, 2H, J=8.2 Hz, H-3); EI-Mass *m/z*: 310.1 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{18}H_{18}N_2O_3$ $[M]^+$; 310.13 Found: 310.13; Anal. Calcd. For $C_{18}H_{18}N_2O_3$: C, 69.67; H, 5.84; N, 9.08; O, 15.42. Found: C, 69.66; H, 5.85; N, 9.03; O, 15.47; λ_{max} = 303 nm

UN-15

Yield: 86%, IR (KBr) cm^{-1}: 3471.5 and 3352.2 (-NH_2, aryl), 3259.7 (N-H, amide), 1652 (C=O, amide), 1510.2 (C=N, imine), 1276.8 (C-N, amide, Aryl amine); ^{1}H NMR (300MHz, DMSO-d_6): δ 10.06 (s, 1H, NH), 7.66 (overlapping m, 3H, H-2', H-6', H-7), 7.39 (overlapping m, 3H, H-4, H-5, H-6), 6.59 (d, 2H, *J* = 8.7 Hz, H-3', H-5'), 5.70 (s, 2H, NH_2), 3.09 (m, 2H, H-2) 2.97 (m, 2H, H-3); EI-Mass *m/z*: 265.0 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{16}H_{15}N_3O$: 265.12 Found 265.12; Anal. Calcd. For $C_{16}H_{15}N_30$: C, 72.43; H, 5.70; N, 15.84; O, 6.03 Found: C, 72.43; H, 5.70; N, 15.84; O, 6.03; λ_{max} = 320 nm

UN-1-16

Yield: 97%, IR (KBr) cm^{-1}: 3227.6 (NH, amide), 1665.7(C=O, amide), 1600.5 (C=N, imine), 1376 (C-N, amide); ^{1}H NMR (300MHz,DMSO-d_6): δ 10.38 (s, 1H, NH), 7.70 (dd, 1H, H-7), 7.37 (d, 2H, J = 7.8 Hz, H-4, H-5), 7.32 (t, 3H, H-3', H-5', H-6), 7.22 (t, 2H, H-2', H-6'), 7.19 (s,1H, H-4'), 3.98 (s, 2H, -CH_2), 3.10 (m, 2H, H-2), 2.85(m, 2H, H-3); EI-Mass *m/z*: 264.1 $[M]^+$; HREI-MS:

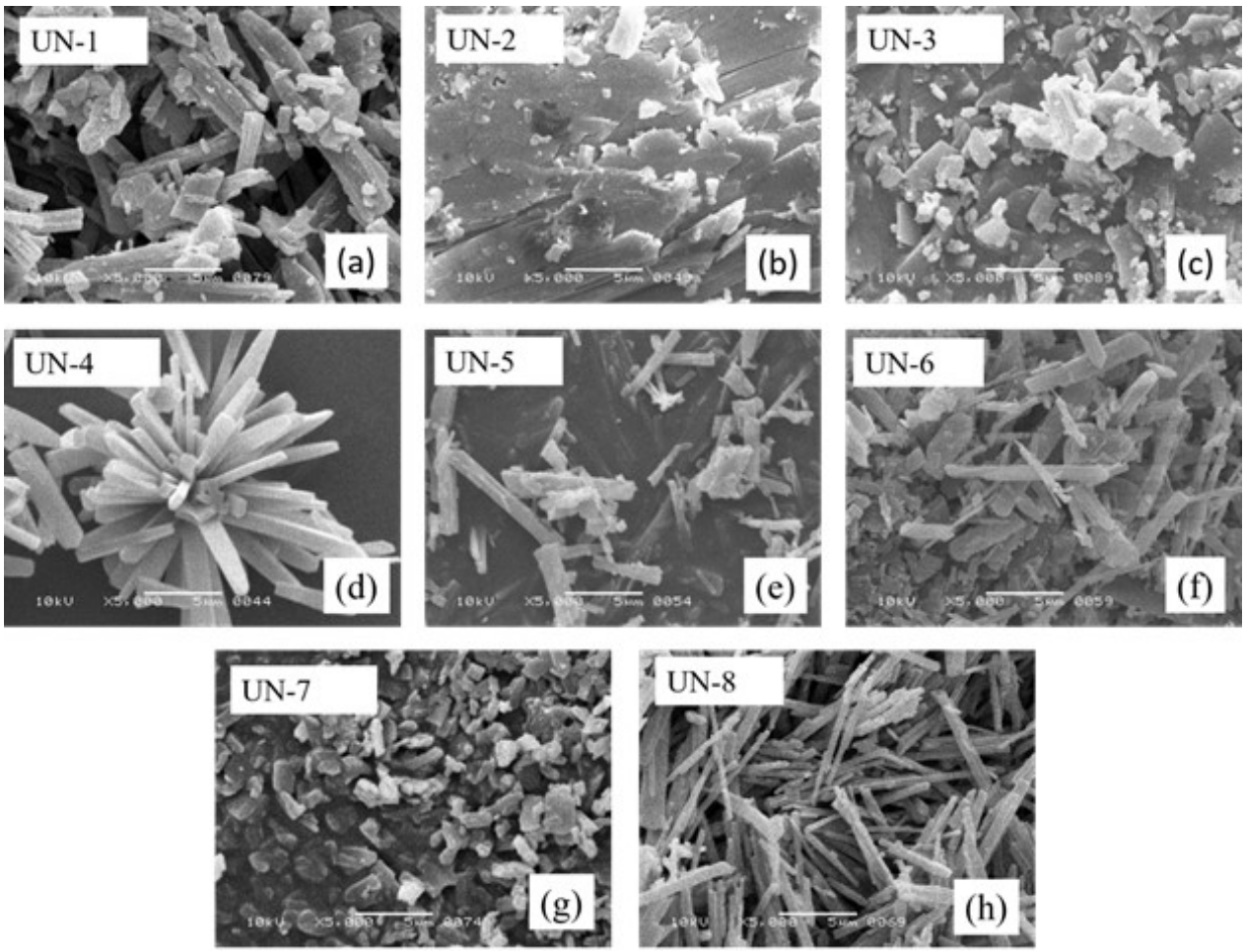


Fig. 2: Scanning Electron Micrographs of the Synthesized Derivatives: (a) UN-1 (b) UN-2 (c) UN-3(d) UN-4 (e) UN-5 (f) UN-6 (g) UN-7 (h) UN-8

m/z Calcd for $C_{17}H_{16}N_2O$ $[M]^+$ 264.12 Found 264.12; Anal. Calcd. For $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60; O, 6.05. Found: C, 77.22; H, 6.13; N, 10.65; O, 6.00; λ_{max} = 310 nm

UN-17

Yield: 90%, IR (KBr) cm^{-1}: IR (KBr) cm^{-1}: 3463.1 (N-H, amide), 3268.8 and 3155.4 (-NH_2, amide), 1695 (C=O, amide), 1585 (C=N, imine), 1285.2 (C-N, amide). ^{1}H NMR (300MHz,DMSO-d_6): δ 9.16 (s, 1H, NH), 7.75 (d, 1H, H-7), 7.73 (dd, 2H, H-5, H-6), 7.28 (m, H-4), 6.41 (s, 2H, NH_2), 3.04 (t, 2H, H-2), 2.73 (t, 2H, H-3), EI-Mass *m/z*: 189.1 $[M]^+$; HREI-MS: *m/z* calcd for $C_{18}H_{11}N_3O$: 189.09 found 189.09; Anal. Calcd. For $C_{18}H_{11}N_3O$: C, 63.48; H, 5.86; N, 22.21; O, 8.46 Found: C, 63.50; H, 5.84; N, 22.24; O, 8.43 λ_{max} = 309 nm

SEM Data

The SEM micrographs of the synthesized derivatives listed in table 1 were presented in fig. 2 and fig. 3. These micrographs revealed interesting morphological features. Remarkably, all the compounds appeared crystalline with either rod or plate type morphology. The compound UN-1 appeared as aggregates of anisotropic, rod like large crystalline particles with sharp edges (fig. 2(a)). The width of the rods was around three micron and the length ranging several nanometers giving rise to a high aspect ratio. As illustrated in fig. 1(b), the compound UN-2 was comprised of irregular, non-spherical platelet-shaped particles with their size ranges in few microns. Clusters of small particles with sizes in nanometer range are also visible which are likely formed due to the fracturing of large plates or are likely due to the impurities in the sample.

The outcomes of anti-mycobacterial study against MTB H37Rv, INH-Sensitive and INH-Resistant were presented in table 2. According to the findings, INH was found to be active against MTB H37Rv at 0.2, 0.5 and 1.0 µg/mL and inactive below 0.2 µg/mL, and active at 0.2, 0.5 and 1.0 µg/mL against INH-S INH-R and inactive below 0.2 µg/mL and inactive at any concentration against INH-R Compound UN-I was found active against INH-S below 0.2 µg/mL (0.1µg/mL)showing that compound UN-I was equally active but more potent than standard drug isoniazid. In the mycobacterium tuberculosis strains in

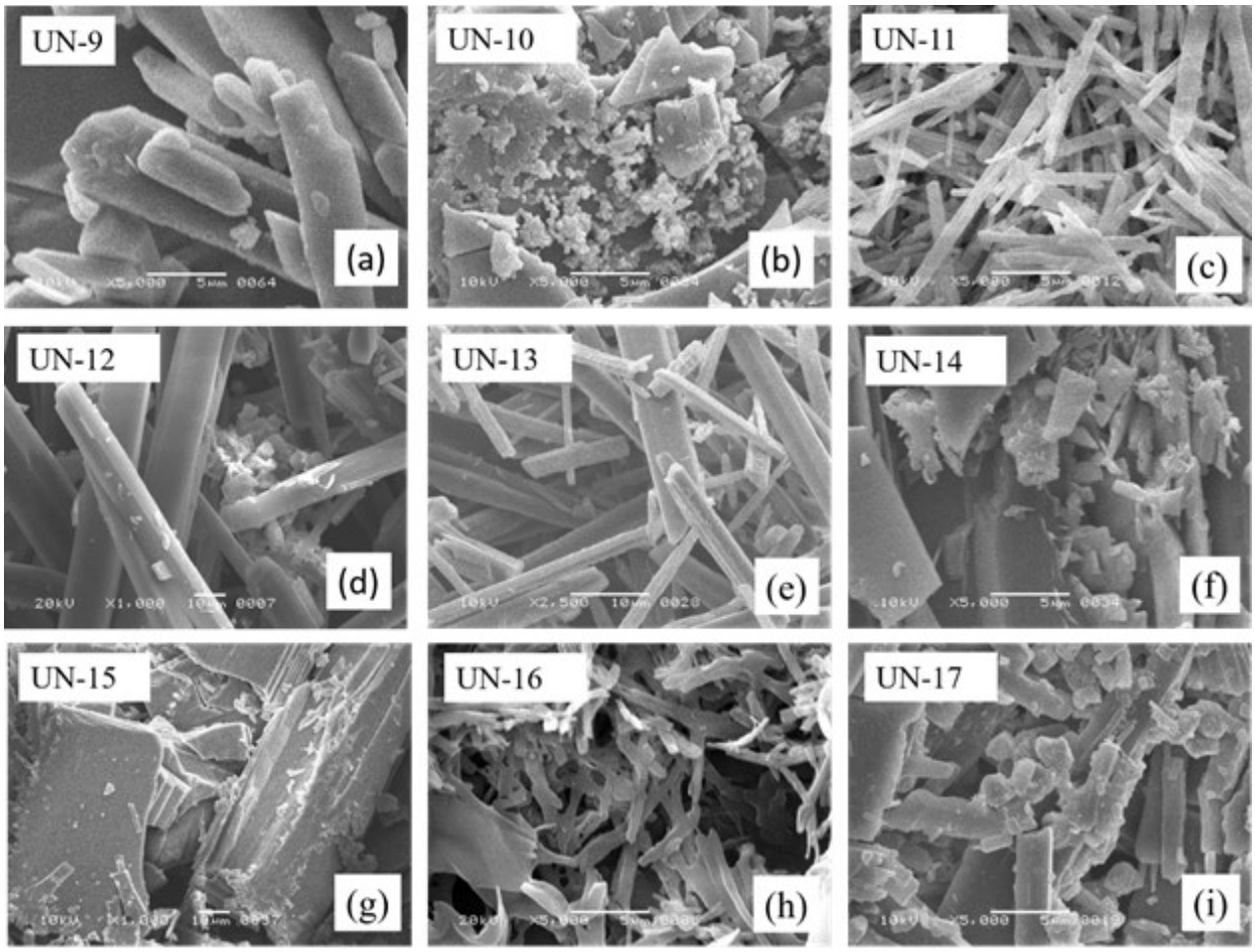


Fig. 3: Scanning Electron Micrographs of the Synthesized Derivatives: (a) UN-9 (b) UN-10 (c) UN-11(d) UN-12 (e) UN-13 (f) UN-14 (g) UN-15 (h) UN-16 (i) UN-17

which isoniazid is completely ineffective at all concentrations (INH-R), compound UN-1 has been found to be effective against INH-R strain at a minimum concentration of 0.1µg / mL.

DISCUSSION

Synthesis

Synthesis of all compounds was successfully achieved using general organic synthetic pathways. The structures of all the synthesized compounds were identified and fully characterized by ^{1}H- NMR, MS and HRMS, FTIR and UV-vis spectroscopy (please refer to table. 1) along with SEM studies. 1-indanyl isoniazid Schiff base (UN-1) was prepared by functionalization of INH at N-2 using standard methods. Typically, treatment of INH with the 1-indanone (a ketone) in ethanol at 80^{0}C using glacial acetic acid as catalyst led to the corresponding Schiff base. Other hydrazide Schiff base derivatives (UN-2 to 17) were obtained by treatment of the corresponding hydrazides with 1-indanone at same temperature using same solvent and catalyst.

SEM studies

Particle morphology and size are arguably the most important physical characteristics of drug that can affect the bulk properties, processability, stability, appearance, area of application, and performance of the end product. Therefore, we decided to explore the morphological features of the synthesized derivatives using Scanning Electron Microscopy (SEM).The technique was selected because of its effectiveness as an investigative tool that provided information about the morphologies and particle size distributions of a wide range of micro and nanosized materials such as drugs. In our case, it was anticipated that SEM can help identifying the existence of crystalline morphology and providing morphological information at the micro- and nanometer scale. All the samples were coated with a thin layer of gold to prevent charge build-up during imaging Similar to the compound UN-1 (fig. 2(a)), the morphologies of the compounds UN-3, UN-4 and UN-5 (table 1) also appeared as anisotropic, rod like crystalline particles (fig. 2(c), (d) and (e), respectively). The particles comprise of sharp edges and the size distribution is quite narrow. The length of the rods is

several microns and their thickness is in the submicron range, resulting in a considerably high aspect ratio. The morphologies of the compounds UN-6, UN-7, UN-8 and UN-9 are shown in fig. 2(f), (g), (h), and (i), respectively. All the four compounds exhibit non-spherical morphology comprising, plate-like particles with sharp edges. The size of the plates is of several microns with a rather broad size distribution. The plates have a tendency of stacking as evident from the appearance of lamellar structures.

The SEM micrographs of the compound UN-10 showed in fig. 3(a) reveals rod-like polydispersed particles of several micron lengths. The morphology is somewhat similar to the morphology of the compound UN-1 showed earlier in fig. 2(a). In the case of UN-11, however, platy crystals are visible in the form of bundles of lamellar sheets that stack over each other (fig. 3(b)). The compounds UN-12 and UN-16 both exhibited an irregular shaped flat particles (fig. 3 (c) and (g)). For the compounds UN-13, UN-14, UN-15 and UN-17, predominantly rod like structures are visible (fig. 3). The morphology is somewhat similar to the morphologies observed for UN-3, UN-4 and UN-5 (fig. 2(c), (d) and (e)).

In conclusion, the morphological data obtained from SEM revealed the existence of crystalline particles for all the compounds explored. The particles were anisotropic with their morphology ranging from platelets to rod shaped particles, in some cases, an apparent tendency of stacking. The sizes of the particles are in the micron range, although nanostructures are also visible.

Anti-tubercular activity

The results present that the synthesized 1-indanyl isoniazide derivative (UN-1) is more active than isoniazid. The antimicrobial activity of isoniazid (INH) is, likely due to its ability to inhibit mycolic acid synthesis, which interferes with cell wall synthesis, thereby producing a bactericidal effect (Oliveira *et al*., 2017). It has been previously studied that the incorporation of lipophilic moieties into the framework of INH can increase permeation of the drug into bacterial cells, thereby enhancing the anti-TB activity (Hu *et al*., 2017). Here in UN-1, Indanyl ring substitution on isoniazid improves lipophilicity, which increases drug penetration across the mycobacterial cell. Moreover, rapid drug resistance developed by M. tuberculosis is generally due to decreased drug permeability induced by the bacteria by thickening their cell wall so that only lipophilic derivatives can penetrate such cell wall (Vergne *et al*., 2000). In addition, acetylation greatly reduces the therapeutic activity of the drug (INH). It is thought that any chemical modification which can prevent acetylation of INH may be useful for proper drug action.

The structure of compound UN-1 represents a lipophilic modification of isoniazid in which the hydrazine moiety has been chemically blocked by schiff base formation with an indanyl moiety on N2 of isoniazide molecule (Hearn and Cynamon, 2004). This structural modification results in prevention of the de-activation process of enzymic N2-acetylation and also enhances the liposolubility of the parent compound (INH) thereby facilitating the entry of this molecules through lipid-enriched bacterial cell wall (Patole *et al*., 2006). Inactivity of the UN-2 compound (which has nicotinyl hydrazide moiety) also makes it clear that only the moiety of isonicotinyl hydrazide is essential for anti-tubercular activity whereas its other isomeric form (Nicotinyl hydrazide) is inactive.

Compounds UN-3- 6 (which have benzene ring and alkyl substituted benzene ring), UN- 7-12 (which have halogen containing benzene ring), UN-13 (with phenolic group), UN-14 (with methoxy benzene), UN-15 (with amino benzene group), UN-16 (with benzyl group) and UN-17 (with amino group) along with indanyl substitution may have greater lipophilicity than isoniazid. But they did not have the characteristic chromophore of isoniazid and were found to be inactive.

CONCLUSION

A 1-indanyl isoniazid Schiff base along with a series of sixteen other hydrazide Schiff bases were successfully synthesized and evaluated for their ability to inhibit M.tb H37Rv strain growth. The high potency and activity was observed in 1-indanyl isoniazid Schiff base (UN-1), possibly due to chemical modification of the hydrazine unit of isoniazid with a lipophilic indanyl group that blocks acetylation, while maintaining strong antimycobacterial action, which has the potential to improve clinical outcomes and reduce the emergence in patients of acquired isoniazid resistance. The prime goal of our study was to investigate such chemical modification. In this regard, exploration. The present research offered a novel isoniazid Schiff base derivative for further exploration as new anti-mycobacterial agent.

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